

What Is Claimed Is:

1. A protease inhibitor comprising the sequence:

X¹-Val-Cys-Ser-Glu-Gln-Ala-Glu-X²-Gly-X³-
Cys-Arg-Ala-X⁴-X⁵-X⁶-X⁷-Trp-Tyr-Phe-Asp-
Val-Thr-Glu-Gly-Lys-Cys-Ala-Pro-Phe-X⁸-
Tyr-Gly-Gly-Cys-X⁹-X¹⁰-X¹¹-X¹²-Asn-Asn-Phe-
Asp-Thr-Glu-Glu-Tyr-Cys-Met-Ala-Val-Cys-
Gly-Ser-Ala-Ile,

wherein:

X¹ is selected from Glu-Val-Val-Arg-Glu-, Asp, or Glu;

X² is selected from Thr, Val, Ile and Ser;

X³ is selected from Pro and Ala;

X⁴ is selected from Arg, Ala, Leu, Gly, or Met;

X⁵ is selected from Ile, His, Leu, Lys, Ala, or Phe;

X⁶ is selected from Ser, Ile, Pro, Phe, Tyr, Trp, Asn, Leu, His, Lys, or Glu;

X⁷ is selected from Arg, His, or Ala;

X⁸ is selected from Phe, Val, Leu, or Gly;

X⁹ is selected from Gly, Ala, Lys, Pro, Arg, Leu, Met, or Tyr;

X¹⁰ is selected from Ala, Arg, or Gly;

X¹¹ is selected from Lys, Ala, or Asn;

X¹² is selected from Ser, Ala, or Arg;

provided that:

when X⁴ is Arg, X⁶ is Ile;

when X⁹ is Arg, X⁴ is Ala or Leu; when X⁹ is Tyr, X⁴ is Ala or X⁵ is His; and

either X⁵ is not Ile; or X⁶ is not Ser; or X⁹ is not Leu, Phe, Met, Tyr, or Asn; or X¹⁰ is not Gly; or X¹¹ is not Asn; or X¹² is not Arg.

2. A protease inhibitor comprising the sequence:

X¹-Val-Cys-Ser-Glu-Gln-Ala-Glu-Thr-Gly-
Pro-Cys-Arg-Ala-X²-X³-X⁴-Arg-Trp-Tyr-Phe-
Asp-Val-Thr-Glu-Gly-Lys-Cys-Ala-Pro-Phe-
Phe-Tyr-Gly-Gly-Cys-X⁵-Gly-Asn-Arg-Asn-

200504-40992001

Asn-Phe-Asp-Thr-Glu-Glu-Tyr-Cys-Met-Ala-
Val-Cys-Gly-Ser-Ala-Ile,

wherein:

X¹ is selected from Glu-Val-Val-Arg-Glu-, Asp, or Glu;

X² is selected from Ala, Leu, Gly, or Met;

X³ is selected from Ile, His, Leu, Lys, Ala, or Phe;

X⁴ is selected from Ser, Ile, Pro, Phe, Tyr, Trp, Asn, Leu, His, Lys, or Glu;

X⁵ is selected from Gly, Ala, Lys, Pro, Arg, Leu, Met, or Tyr;

provided that:

when X⁵ is Arg, X² is Ala or Leu; when X⁵ is Tyr, X² is Ala or X³ is His; and

either X³ is not Ile; or X⁴ is not Ser; or X⁵ is not Leu, Phe, Met, Tyr, or Asn.

3. A protease inhibitor comprising the sequence:

Glu-Val-Val-Arg-Glu-Val-Cys-Ser-Glu-Gln-
Ala-Glu-Thr-Gly-Pro-Cys-Arg-Ala-X¹-X²-X³-
Arg-Trp-Tyr-Phe-Asp-Val-Thr-Glu-Gly-Lys-
Cys-Ala-Pro-Phe-Phe-Tyr-Gly-Gly-Cys-X⁴-
Gly-Asn-Arg-Asn-Asn-Phe-Asp-Thr-Glu-Glu-
Tyr-Cys-Met-Ala-Val-Cys-Gly-Ser-Ala-Ile,

wherein:

X¹ is selected from Ala, Leu, Gly, or Met;

X² is selected from Ile, His, Leu, Lys, Ala, or Phe;

X³ is selected from Ser, Ile, Pro, Phe, Tyr, Trp, Asn, Leu, His, Lys, or Glu;

X⁴ is selected from Gly, Arg, Leu, Met, or Tyr;

provided that:

when X¹ is Ala, X² is Ile, His, or Leu;

when X¹ is Leu, X² is Ile or His;

when X¹ is Leu and X² is Ile, X³ is not Ser;

when X¹ is Gly, X² is Ile;

when X⁴ is Arg, X¹ is Ala or Leu;

when X⁴ is Tyr, X¹ is Ala or X² is His; and

either X¹ is not Met, or X² is not Ile, or X³ is not Ser, or X⁴ is not Gly.

20076604-021902

4. A protease inhibitor according to claim 1, wherein at least two amino acid residues selected from the group consisting of X⁴, X⁵, X⁶, and X⁷ differ from the residues found in the naturally occurring sequence of KPI.

5. A protease inhibitor according to claim 1, wherein X¹ is Asp or Glu, X² is Thr, X³ is Pro, and X¹² is Ser.

6. A protease inhibitor according to claim 5, wherein X¹ is Glu, X² is Thr, X³ is Pro, X⁴ is Met, X⁵ is Ile, X⁶ is Ser, X⁷ is Arg, X⁸ is Phe, X⁹ is Gly, X¹⁰ is Gly, and X¹¹ is Asn.

7. A protease inhibitor according to claim 5, wherein X¹ is Asp, X² is Thr, X³ is Pro, X⁴ is Arg, X⁵ is Ile, X⁶ is Ile, X⁷ is Arg, X⁸ is Val, X⁹ is Arg, X¹⁰ is Ala, and X¹¹ is Lys.

8. A protease inhibitor according to claim 1, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, X³ is Pro, X⁴ is Met, X⁵ is Ile, X⁶ is Ser, X⁷ is Arg, X⁸ is Phe, X⁹ is Gly, X¹⁰ is Gly, X¹¹ is Asn, and X¹² is Ala.

9. A protease inhibitor according to claim 1, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, X³ is Pro, X⁴ is Met, X⁵ is Ile, X⁶ is Ser, X⁷ is Arg, X⁸ is Phe, X⁹ is Gly, X¹⁰ is Gly, X¹¹ is Ala, and X¹² is Arg.

10. A protease inhibitor according to claim 1, wherein X¹ is Glu, X² is Thr, X³ is Pro, X⁴ is Met, X⁵ is Ile, X⁶ is Ser, X⁷ is Arg, X⁸ is Phe, X⁹ is Gly, X¹⁰ is Ala, X¹¹ is Asn, and X¹² is Arg.

11. A protease inhibitor according to claim 1, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, X³ is Pro, X⁴ is Met, X⁵ is Ile, X⁶ is Ser, X⁷ is Arg, X⁸ is Phe, X⁹ is Gly, X¹⁰ is Arg, X¹¹ is Asn, and X¹² is Arg.

20076604.031902

12. A protease inhibitor according to claim 1, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, X³ is Pro, X⁴ is Met, X⁵ is Ile, X⁶ is Ser, X⁷ is Arg, X⁸ is Val, Leu, or Gly, X⁹ is Gly, X¹⁰ is Gly, X¹¹ is Asn, and X¹² is Arg.

13. A protease inhibitor according to claim 1, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, X³ is Pro, X⁴ is Met, X⁵ is Ile, X⁶ is Ser, X⁷ is Ala, X⁸ is Phe, X⁹ is Gly, X¹⁰ is Gly, X¹¹ is Asn, and X¹² is Arg.

14. A protease inhibitor according to claim 1, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, Val, or Ser, X³ is Pro, X⁴ is Ala or Leu, X⁵ is Ile, X⁶ is Tyr, X⁷ His, X⁸ is Phe, X⁹ is Gly, X¹⁰ is Gly, X¹¹ is Ala, and X¹² is Arg.

15. A protease inhibitor according to claim 14, wherein X² is Thr, and X⁴ is Ala.

16. A protease inhibitor according to claim 14, wherein X² is Thr, and X⁴ is Leu.

17. A protease inhibitor according to claim 14, wherein X² is Val, and X⁴ is Ala.

18. A protease inhibitor according to claim 14, wherein X² is Ser, and X⁴ is Ala.

19. A protease inhibitor according to claim 14, wherein X² is Val, and X⁴ is Leu.

20. A protease inhibitor according to claim 14, wherein X² is Ser, and X⁴ is Leu.

21. A protease inhibitor according to claim 1, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, X³ is Pro, X⁴ is Leu, X⁵ is Phe, X⁶ is Lys, X⁷ is Arg, X⁸ is Phe, X⁹ is Gly, X¹⁰ is Gly, X¹¹ is Ala, and X¹² is Arg.

202504091007

22. A protease inhibitor according to claim 1, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, X³ is Pro, X⁴ is Leu, X⁵ is Phe, X⁶ is Lys, X⁷ is Arg, X⁸ is Phe, X⁹ is Tyr, X¹⁰ is Gly, X¹¹ is Ala, and X¹² is Arg.

23. A protease inhibitor according to claim 1, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, X³ is Pro, X⁴ is Leu, X⁵ is Phe, X⁶ is Lys, X⁷ is Arg, X⁸ is Phe, X⁹ is Leu, X¹⁰ is Gly, X¹¹ is Ala, and X¹² is Arg.

24. A protease inhibitor according to claim 2, wherein X¹ is Glu, X² is Met, X³ is Ile, X⁴ is Ile, and X⁵ is Gly.

25. A protease inhibitor according to claim 3, wherein X¹ is Met, X³ is Ser, and X⁴ is Gly.

26. A protease inhibitor according to claim 25, wherein X² is selected from His, Ala, Phe, Lys, and Leu.

27. A protease inhibitor according to claim 26, wherein X² is His.

28. A protease inhibitor according to claim 27, wherein X² is Ala.

29. A protease inhibitor according to claim 27, wherein X² is Phe.

30. A protease inhibitor according to claim 27, wherein X² is Lys.

31. A protease inhibitor according to claim 27, wherein X² is Leu.

32. A protease inhibitor according to claim 3, wherein X¹ is Met, X² is Ile, and X⁴ is Gly.

20075604-021902

33. A protease inhibitor according to claim 32, wherein X³ is Ile.

34. A protease inhibitor according to claim 32, wherein X³ is Pro.

35. A protease inhibitor according to claim 32, wherein X³ is Phe.

36. A protease inhibitor according to claim 32, wherein X³ is Tyr.

37. A protease inhibitor according to claim 32, wherein X³ is Trp.

38. A protease inhibitor according to claim 32, wherein X³ is Asn.

39. A protease inhibitor according to claim 32, wherein X³ is Leu.

40. A protease inhibitor according to claim 32, wherein X³ is Lys.

41. A protease inhibitor according to claim 32, wherein X³ is His.

42. A protease inhibitor according to claim 32, wherein X³ is Glu.

43. A protease inhibitor according to claim 3, wherein X¹ is Ala.

44. A protease inhibitor according to claim 43, wherein X² is Ile.

45. A protease inhibitor according to claim 44, wherein X³ is Phe, and X⁴ is Gly.

1006604-031902

46. A protease inhibitor according to claim 44, wherein X³ is Tyr, and X⁴ is Gly.

47. A protease inhibitor according to claim 44, wherein X³ is Trp, and X⁴ is Gly.

48. A protease inhibitor according to claim 44, wherein X³ is Ser or Phe, and X⁴ is Arg or Tyr.

49. A protease inhibitor according to claim 43, wherein X² is His or Leu, X³ is Phe, and X⁴ is Gly.

50. A protease inhibitor according to claim 3, wherein X¹ is Leu.

51. A protease inhibitor according to claim 50, wherein X² is His, X³ is Asn or Phe, and X⁴ is Gly.

52. A protease inhibitor according to claim 50, wherein X² is Ile, X³ is Pro, and X⁴ is Gly.

53. A protease inhibitor according to claim 3, wherein X¹ is Gly, X² is Ile, X³ is Tyr, and X⁴ is Gly.

54. A protease inhibitor according to claim 3, wherein X¹ is Met, X² is His, X³ is Ser, and X⁴ is Tyr.

55. An isolated DNA molecule comprising a DNA sequence encoding a protease inhibitor according to claim 1.

56. An isolated DNA molecule according to claim 55, operably linked to a regulatory sequence that controls expression of the coding sequence in a host cell.

57. An isolated DNA molecule according to claim 56, further comprising a DNA sequence encoding a secretory signal peptide. .

206720-409200F

58. An isolated DNA molecule according to claim 57, wherein said secretory signal peptide comprises the signal sequence of yeast alpha-mating factor.

59. A host cell transformed with a DNA molecule according to claim 55.

60. A host cell according to claim 59, wherein said host cell is *E. coli* or a yeast cell.

61. A host cell according to claim 60, wherein said host cell is *Saccharomyces cerevisiae*.

62. A method for producing a protease inhibitor, comprising the steps of culturing a host cell according to claim 59 and isolating and purifying said protease inhibitor.

63. A pharmaceutical composition, comprising a protease inhibitor according to claim 1, together with a pharmaceutically acceptable sterile vehicle.

64. A method of treatment of a clinical condition associated with increased activity of one or more serine proteases, comprising administering to a patient suffering from said clinical condition an effective amount of a pharmaceutical composition according to claim 63.

65. The method of treatment of claim 64, wherein said clinical condition is blood loss during surgery.

66. A method for inhibiting the activity of serine proteases of interest in a mammal comprising administering a therapeutically effective dose of a pharmaceutical composition according to claim 63.

67. The method of claim 66, wherein said serine proteases are selected from the group consisting of:

206T20-4099Z00T

kallikrein; chymotrypsins A and B; trypsin; elastase; subtilisin; coagulants and procoagulants, particularly those in active form, including coagulation factors such as factors VIIa, IXa, Xa, XIa, and XIIa; plasmin; thrombin; proteinase-3; enterokinase; acrosin; cathepsin; urokinase; and tissue plasminogen activator.

68. A protease inhibitor comprising the sequence:

X¹-Val-Cys-Ser-Glu-Gln-Ala-Glu-X²-Gly-Pro-
Cys-Arg-Ala-X³-X⁴-X⁵-X⁶-Arg-Trp-Tyr-Phe-
Asp-Val-Thr-Glu-Gly-Lys-Cys-Ala-Pro-Phe-
Phe-Tyr-Gly-Gly-Cys-X⁷-Gly-Asn-Arg-Asn-
Asn-Phe-Asp-Thr-Glu-Glu-Tyr-Cys-Met-Ala-
Val-Cys-Gly-Ser-Ala-Ile,

wherein:

X¹ is selected from Glu-Val-Val-Arg-Glu-, Asp, or Glu;

X² is selected from Thr, Val, Ile and Ser;

X³ is selected from Arg, Ala, Leu, Gly, or Met;

X⁴ is selected from Ile, His, Leu, Lys, Ala, or Phe;

X⁵ is selected from Ser, Ile, Pro, Phe, Tyr, Trp, Asn, Leu, His, Lys, or Glu;

X⁶ is selected from Arg, His, or Ala; and

X⁷ is selected from Gly, Ala, Lys, Pro, Arg, Leu, Met, or Tyr.

69. A protease inhibitor according to claim 68, wherein at least two amino acid residues selected from the group consisting of X³, X⁴, X⁵, and X⁶ differ from the residues found in the naturally occurring sequence of KPI.

70. A protease inhibitor according to claim 68, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, Val, or Ser, X³ is Ala or Leu, X⁴ is Ile, X⁵ is Tyr, X⁶ is His and X⁷ is Gly.

71. A protease inhibitor according to claim 70, wherein X² is Thr, and X³ is Ala.

10076604-021902

72. A protease inhibitor according to claim 70, wherein X² is Thr, and X³ is Leu.

73. A protease inhibitor according to claim 70, wherein X² is Val, and X³ is Ala.

74. A protease inhibitor according to claim 70, wherein X² is Ser, and X³ is Ala.

75. A protease inhibitor according to claim 70, wherein X² is Val, and X³ is Leu.

76. A protease inhibitor according to claim 70, wherein X² is Ser, and X³ is Leu.

77. A protease inhibitor according to claim 68, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, X³ is Leu, X⁴ is Phe, X⁵ is Lys, X⁶ is Arg and X⁷ is Gly.

78. A protease inhibitor according to claim 68, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, X³ is Leu, X⁴ is Phe, X⁵ is Lys, X⁶ is Arg and X⁷ is Tyr.

79. A protease inhibitor according to claim 68, wherein X¹ is Glu-Val-Val-Arg-Glu-, X² is Thr, X³ is Leu, X⁴ is Phe, X⁵ is Lys, X⁶ is Arg and X⁷ is Leu.

10076504-031902